cells

MDPI

Editorial

Context-Dependent Phenotypes in Skeletal Muscle Regeneration, Metabolism, and Disease

Wan Lee [1,2]

[1] Department of Biochemistry, Dongguk University College of Medicine, 123 Dongdae-ro, Gyeongju 38066, Republic of Korea; wanlee@dongguk.ac.kr; Tel.: +82-54-770-2409
[2] Section of Molecular and Cellular Medicine, Medical Institute of Dongguk University, Gyeongju 38066, Republic of Korea

1. Introduction

Every phenotype reported in muscle research is a difference, and every difference has a denominator. Whether a protein is said to promote or impair myogenesis, whether a transcriptional program is classified as excessive or deficient, and whether a kinase is described as protective or harmful all depend on what the measurement was compared with. In most fields, the comparator is chosen once and seldom revisited. In muscle, it deserves more attention than it usually receives, because muscle is rarely at rest in the sense that a control group implies. It regenerates after injury, remodels under loading and unloading, switches fuel with nutritional state and passes through a series of distinct states as myoblasts become myotubes. A resting, uninjured, sedentary control is a legitimate reference, but it is one reference among several, and it is often not the one that answers the question being asked.

The seven contributions to the Special Issue "Advances in Muscle Research in Health and Disease—2nd Edition" illustrate this point from unusually different directions. Five are original articles and two are reviews, published between 2024 and 2026, and together they continue the scope set out for the first edition [1]. They range from dystrophic mouse and human muscle (Contribution 1) and the gastrointestinal smooth muscle of gerbils returned from orbit (Contribution 2) to cultured myoblasts under genetic, nutritional, and chemical challenge (Contributions 3 and 4), the metabolic signaling of skeletal muscle in type 2 diabetes (Contribution 5), the triad junction of dysferlin-deficient fibers (Contribution 6), and the staging of myotube fusion in vitro (Contribution 7). Superficially, they share only a tissue. Read together, each turns at a decisive point on the choice of a reference state.

2. The Resting Control Is Not Neutral

Perin et al. (Contribution 1) state the problem most directly. Dystrophic muscle degenerates and regenerates continuously, so its stem cells are always partway through a regenerative response that an uninjured wild-type control never undergoes. A comparison of the two therefore mixes disease with regeneration. The authors calibrate dystrophic data against normal regeneration trajectories at matched lineage state and niche. In activated stem cells, extracellular matrix induction was excessive in mdx relative to wild-type cells (standardized difference 1.431) and the MYC-associated response was attenuated (standardized difference −0.731). Calibration showed that matrix induction is a normal transient program that becomes excessive in dystrophy, and that reduced oxidative phosphorylation transcription, which would conventionally be read as impaired oxidative metabolism, may instead reflect insufficient suppression relative to regenerating muscle. The authors

Check for updates

Received: 22 October 2026
Accepted: 19 November 2026
Published: 24 November 2026

Copyright: © 2026 by the author. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license.

present this second reading as a hypothesis generated by the framework rather than a mechanistic conclusion.

Their audit of published claims is informative for what it did not find. Of 79 claims drawn from seven earlier studies, 48 could be evaluated. Only nine of these were confirmed unchanged, yet the authors stress that most were not negated. They were refined, or became conditional on spatial context or tissue composition. The authors also report that the mouse MYC signal was not reproduced in a tissue-matched human comparison, and they label the supportive pattern obtained with unmatched controls as confounded by tissue. These results do not imply that earlier findings were simply incorrect. Instead, they show that their interpretation may change when an explicit physiological reference is introduced.

Samoilenko et al. (Contribution 2) built the same logic into an animal design. Gerbils flown for 12 days on the Foton M-3 mission were compared not only with vivarium controls but also with a synchronous ground control, housed for the same period in the flight hardware under all conditions except weightlessness. Integrin alpha-5 immunoreactivity in gastric smooth muscle fell to 2.46% of the field after flight, against 10.79% in vivarium animals and 9.09% in ground controls, and the jejunum showed the same pattern. Because the ground control did not differ from the vivarium group, the design supports attributing the difference to the flight condition rather than to confinement in the hardware alone. The second reference therefore strengthens attribution of the observed difference to the flight condition. The authors acknowledge that the data are immunohistochemical, obtained from archived tissue and without functional measurements. The animals were examined 21 h after landing, so the state captured is spaceflight followed by early reloading rather than weightlessness itself.

3. One Perturbation, Opposite Readings

The cultured myoblast is often treated as a single reference system, but it is a sequence of states. Nguyen et al. (Contribution 3) reduced Calponin 3 by about half in C2C12 myoblasts and observed an increase of about 1.7-fold in filamentous actin, nuclear accumulation of YAP1, larger S and G2/M fractions, and faster proliferation, followed by reduced MyoD, myogenin, and myosin heavy chain and impaired fusion. Nuclear YAP supports myoblast proliferation, whereas reduced nuclear YAP activity accompanies the transition to differentiation [2–4]. The same knockdown would therefore be described as pro-proliferative at the progenitor stage but anti-differentiative at the myotube stage. Both effects are necessary to interpret the phenotype, and each is incomplete without the stage at which it was measured. The authors note that external mechanical cues, which act on YAP1 through the same cytoskeleton, were not examined.

Noboa Carrasco et al. (Contribution 4) found that the nucleotide formulation NooQ5 favored proliferation at 500 mg/L and differentiation at 100 mg/L, a dose dependence that again separates the two phases. Their design deserves note for a further reason. NooQ5 was compared with creatine monohydrate as an active reference in addition to untreated cells, and with N-acetylcysteine and insulin-like growth factor 1 as positive controls in the oxidative and barium chloride injury models. Comparison with creatine provided a more informative benchmark than an untreated control alone and allowed the authors to assess whether NooQ5 offered an incremental effect. Under hydrogen peroxide, lactate dehydrogenase release was about 60 U/L with NooQ5 and about 90 U/L with creatine. Whether such differences hold in muscle in vivo is the question the authors identify as the next step.

Bengal and Aviram (Contribution 5) review the metabolic counterpart. Skeletal muscle accounts for about 80% of insulin-stimulated glucose disposal [5,6], and intramyocellular lipid accumulates both in endurance-trained athletes, who are highly insulin-sensitive, and

in patients with type 2 diabetes, who are not [7,8]. The same measurement carries opposite meanings in the two states. The kinase at the center of the review behaves in the same way. Bengal and Aviram describe how p38α enhances mitochondrial oxidative capacity and cooperates with AMP-activated protein kinase during exercise. They also review evidence implicating p38α or p38β in muscle atrophy, depending on the model. In myoblasts, p38α is required for cell-cycle exit [9,10]. The authors state that their own lipid oxidation finding rests on a single model and that the therapeutic strategies they propose remain speculative. Their case for muscle-restricted p38α activation as an exercise mimetic depends accordingly on specifying the state in which the kinase is to be activated.

4. When the Assay Defines the Function

Bloch et al. (Contribution 6) address a protein whose function was, for two decades, largely defined by the assay used to study it. Mutations in dysferlin cause limb girdle muscular dystrophy type 2B, Miyoshi myopathy, and related dysferlinopathies [11–13], and a large body of in vitro membrane repair studies, with laser wounding prominent among them, established it as a component of the membrane repair machinery [14,15]. The authors argue that laser injury also damages the transverse tubules, that truncated dysferlins restore laser-wound repair without restoring muscle histology, and that dysferlin concentrates at the triad junction, where its loss increases Ca2+ leak, reduces the amplitude of Ca2+ transients, and triggers Ca2+-induced Ca2+ release in the form of propagating waves. After osmotic shock injury, wild-type fibers recover 70 to 80% of transient amplitude within 5 min, whereas dysferlin-null fibers recover about 30%. The review is candid that this simple model does not account for several observations, and it closes with open questions, including whether the Ca2+ and repair defects arise from the same mutations. What it shows clearly is that the function assigned to a protein is relative to the readout, and that a readout able to restore one measure of repair without restoring tissue health is a poor reference against which to judge a therapy.

Jeong et al. (Contribution 7) make a related point at the level of differentiation stage. They transferred myoblasts, or immature myotubes on day 2 or day 3 of differentiation, onto host cultures at day 2 or day 3, giving six combinations. Only day 2 cells transferred onto day 3 hosts produced wider myotubes, higher MyoD and fast myosin heavy chain, and greater Ca2+ release and entry, with increased RyR1, SERCA1a, and STIM1, and a fusion index of 70.0% against 54.8% in controls. The authors also report that thicker myotubes in other conditions did not always bring functional gains, so that appearance and function had to be measured separately. The finding bears on a long record in cell therapy. Cell therapy for Duchenne muscular dystrophy, whether with myoblasts, satellite cells, mesoangioblasts, or cells derived from induced pluripotent stem cells, has been limited by poor engraftment and low delivery efficiency [16,17]. Expansion in culture, which is needed to reach therapeutic cell numbers, reduces the regenerative capacity of satellite cells [18], and expanded mesoangioblasts have so far shown safety but minimal efficacy in patients [19]. What the donor cell is, and in which state it meets its host, is part of the reference. The present data are in vitro, and whether the advantage of the day 2 to day 3 pairing holds in host muscle remains to be tested.

5. What This Implies for Muscle Research

Three requests follow from the papers gathered here. First, report the reference state as a primary element of design, including regenerative stage, day of differentiation, loading history, and nutritional state. Where the tissue is in transition, add a physiological comparator to the resting control rather than replacing one with the other. The matched regeneration trajectories of Perin et al. (Contribution 1) and the synchronous ground control

of Samoilenko et al. (Contribution 2) are two forms of the same discipline. Second, report effects by stage rather than issuing a single verdict. A factor that expands progenitors and impairs fusion (Contribution 3), or a nutrient whose effect changes with dose (Contribution 4), is described accurately only when both phases are shown. Third, choose readouts that bear on function, and report the cases in which morphology or a surrogate assay diverges from function (Contributions 6 and 7), because those cases are the most informative.

None of this is new methodology. It is the ordinary practice of comparing like with like, applied to a tissue that rarely stays still long enough for a single control to represent it. These contributions suggest that part of the field's interpretive uncertainty arises from incompletely specified reference states. This is a correctable limitation in experimental design and reporting.

Funding: This work received no external funding.

Acknowledgments: I thank all authors who contributed to this Special Issue, the reviewers whose assessments shaped the published versions, and the editorial staff of *Cells* for their support throughout this Special Issue and its predecessor.

Conflicts of Interest: The author served as Guest Editor of this Special Issue and is a co-author of one of the contributions discussed here (Contribution 3). The author declares no other conflicts of interest.

List of Contributions:

1. Perin, L.; Liu, Y.-Y.; Brent, G.A.; Milanesi, A. Physiological Reference Mapping Reveals Context-Dependent Dysregulation of Regenerative Programs in Duchenne Muscular Dystrophy. *Cells* **2026**, *15*, 1743. https://doi.org/10.3390/cells15191743.
2. Samoilenko, T.; Shishkina, V.; Zolotaryova, S.; Kostin, A.; Alekhnovich, A.; Volodkin, A.; Kireeva, A.; Buchwalow, I.; Tiemann, M.; Atiakshin, D. Study of the Expression of Integrins in Smooth Myocytes of the Gastrointestinal Organs After Space Flight. *Cells* **2026**, *15*, 1730. https://doi.org/10.3390/cells15191730.
3. Nguyen, M.T.; Ly, Q.K.; Ngo, T.H.P.; Lee, W. Calponin 3 Regulates Myoblast Proliferation and Differentiation Through Actin Cytoskeleton Remodeling and YAP1-Mediated Signaling in Myoblasts. *Cells* **2025**, *14*, 142. https://doi.org/10.3390/cells14020142.
4. Noboa Carrasco, K.X.; Andrade Alfaro, C.D.; Cremonese Lubiana, A.L.; Viana Ferreira, R.; Jorge, E.C. NooQ5 Supplementation Promotes Myogenic Differentiation in C2C12 Cells Under Chemical and Oxidative Stress Models. *Cells* **2026**, *15*, 1719. https://doi.org/10.3390/cells15191719.
5. Bengal, E.; Aviram, S. p38α MAPK Regulation of Energy Metabolism in Skeletal Muscle Offers a Therapeutic Path for Type 2 Diabetes. *Cells* **2025**, *14*, 1277. https://doi.org/10.3390/cells14161277.
6. Bloch, R.J.; Muriel, J.; Lukyanenko, V. Dysferlin and the Regulation of Ca2+ Release in Skeletal Muscle. *Cells* **2025**, *14*, 1724. https://doi.org/10.3390/cells14211724.
7. Jeong, S.Y.; Choi, J.H.; Allen, P.D.; Lee, E.H. Immature Skeletal Myotubes Are an Effective Source for Improving the Terminal Differentiation of Skeletal Muscle. *Cells* **2024**, *13*, 2136. https://doi.org/10.3390/cells13242136.

References

1. Lee, W. Advances in Muscle Research in Health and Disease. *Cells* **2024**, *13*, 1694. [CrossRef] [PubMed]
2. Watt, K.I.; Judson, R.; Medlow, P.; Reid, K.; Kurth, T.B.; Burniston, J.G.; Ratkevicius, A.; De Bari, C.; Wackerhage, H. Yap is a novel regulator of C2C12 myogenesis. *Biochem. Biophys. Res. Commun.* **2010**, *393*, 619–624. [CrossRef] [PubMed]
3. Meinhold, M.; Verbrugge, S.; Shi, A.; Schönfelder, M.; Becker, L.; Jaspers, R.T.; Zammit, P.S.; Wackerhage, H. Yap/Taz activity is associated with increased expression of phosphoglycerate dehydrogenase that supports myoblast proliferation. *Cell Tissue Res.* **2024**, *395*, 271–283. [CrossRef] [PubMed]
4. Kwon, Y. YAP/TAZ as Molecular Targets in Skeletal Muscle Atrophy and Osteoporosis. *Aging Dis.* **2024**, *16*, 299–320. [CrossRef] [PubMed]
5. DeFronzo, R.A.; Jacot, E.; Jequier, E.; Maeder, E.; Wahren, J.; Felber, J.P. The effect of insulin on the disposal of intravenous glucose. Results from indirect calorimetry and hepatic and femoral venous catheterization. *Diabetes* **1981**, *30*, 1000–1007. [CrossRef] [PubMed]

6. Coker, M.S.; Coker, R.H. Dietary proteins, amino acids and insulin resistance: A mini review. *Front. Nutr.* **2025**, *12*, 1671286. [CrossRef] [PubMed]
7. Goodpaster, B.H.; He, J.; Watkins, S.; Kelley, D.E. Skeletal muscle lipid content and insulin resistance: Evidence for a paradox in endurance-trained athletes. *J. Clin. Endocrinol. Metab.* **2001**, *86*, 5755–5761. [CrossRef] [PubMed]
8. Mezincescu, A.M.; Rudd, A.; Cheyne, L.; Horgan, G.; Philip, S.; Cameron, D.; van Loon, L.; Whitfield, P.; Gribbin, R.; Hu, M.K.; et al. Comparison of intramyocellular lipid metabolism in patients with diabetes and male athletes. *Nat. Commun.* **2024**, *15*, 3690. [CrossRef] [PubMed]
9. Perdiguero, E.; Ruiz-Bonilla, V.; Gresh, L.; Hui, L.; Ballestar, E.; Sousa-Victor, P.; Baeza-Raja, B.; Jardi, M.; Bosch-Comas, A.; Esteller, M.; et al. Genetic analysis of p38 MAP kinases in myogenesis: Fundamental role of p38α in abrogating myoblast proliferation. *EMBO J.* **2007**, *26*, 1245–1256. [CrossRef] [PubMed]
10. Endo, T. Postnatal skeletal muscle myogenesis governed by signal transduction networks: MAPKs and PI3K-Akt control multiple steps. *Biochem. Biophys. Res. Commun.* **2023**, *682*, 223–243. [CrossRef] [PubMed]
11. Liu, J.; Aoki, M.; Illa, I.; Wu, C.; Fardeau, M.; Angelini, C.; Serrano, C.; Urtizberea, J.A.; Hentati, F.; Hamida, M.B.; et al. Dysferlin, a novel skeletal muscle gene, is mutated in Miyoshi myopathy and limb girdle muscular dystrophy. *Nat. Genet.* **1998**, *20*, 31–36. [CrossRef] [PubMed]
12. Bashir, R.; Britton, S.; Strachan, T.; Keers, S.; Vafiadaki, E.; Lako, M.; Richard, I.; Marchand, S.; Bourg, N.; Argov, Z.; et al. A gene related to Caenorhabditis elegans spermatogenesis factor fer-1 is mutated in limb-girdle muscular dystrophy type 2B. *Nat. Genet.* **1998**, *20*, 37–42. [CrossRef] [PubMed]
13. Poudel, B.H.; Fletcher, S.; Wilton, S.D.; Aung-Htut, M. Limb Girdle Muscular Dystrophy Type 2B (LGMD2B): Diagnosis and Therapeutic Possibilities. *Int. J. Mol. Sci.* **2024**, *25*, 5572. [CrossRef] [PubMed]
14. Bansal, D.; Miyake, K.; Vogel, S.S.; Groh, S.; Chen, C.C.; Williamson, R.; McNeil, P.L.; Campbell, K.P. Defective membrane repair in dysferlin-deficient muscular dystrophy. *Nature* **2003**, *423*, 168–172. [CrossRef] [PubMed]
15. Quinn, C.J.; Cartwright, E.J.; Trafford, A.W.; Dibb, K.M. On the role of dysferlin in striated muscle: Membrane repair, t-tubules and Ca^{2+} handling. *J. Physiol.* **2024**, *602*, 1893–1910. [CrossRef] [PubMed]
16. Mendell, J.R.; Kissel, J.T.; Amato, A.A.; King, W.; Signore, L.; Prior, T.W.; Sahenk, Z.; Benson, S.; McAndrew, P.E.; Rice, R.; et al. Myoblast transfer in the treatment of Duchenne's muscular dystrophy. *N. Engl. J. Med.* **1995**, *333*, 832–838. [CrossRef] [PubMed]
17. Łoboda, A.; Dulak, J. Cell therapy for Duchenne muscular dystrophy: Promises, challenges, and controversies. *Cell Mol. Life Sci.* **2025**, *82*, 356. [CrossRef] [PubMed]
18. Montarras, D.; Morgan, J.; Collins, C.; Relaix, F.; Zaffran, S.; Cumano, A.; Partridge, T.; Buckingham, M. Direct isolation of satellite cells for skeletal muscle regeneration. *Science* **2005**, *309*, 2064–2067. [CrossRef] [PubMed]
19. Cossu, G.; Tonlorenzi, R.; Brunelli, S.; Sampaolesi, M.; Messina, G.; Azzoni, E.; Benedetti, S.; Biressi, S.; Bonfanti, C.; Bragg, L.; et al. Mesoangioblasts at 20: From the embryonic aorta to the patient bed. *Front. Genet.* **2023**, *13*, 1056114, Correction in *Front. Genet.* **2026**, *17*, 1884212. [CrossRef] [PubMed]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.